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Stressor intensity determines antagonistic interactions between species invasion and multiple stressor effects on ecosystem functioning

Siobhan R. Vye, Mark C. Emmerson, Francisco Arenas, Jaimie T. A. Dick and Nessa E. O'Connor

S. R. Vye (svye01@qub.ac.uk), M. C. Emmerson, J. T. A. Dick and N. E. O'Connor, School of Biological Sciences, Medical Biology Centre, Queen's Univ. Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK, and: Queen's Univ. Marine Laboratory, 12-13 The Strand, Portaferry, Co. Down, Northern Ireland, BT22 1PF, UK. MCE, JTAD and NEO also at: Inst. for Global Food Security, Queen's Univ. Belfast, 18-30 Malone Road, Belfast, BT9 5BN, UK. – F. Arenas, CIIMAR, Univ. of Porto, Rua dos Bragas, n.289, PT-4050-123 Porto, Portugal.

Biological invasions, nutrient enrichment and ocean warming are known to threaten biodiversity and ecosystem functioning. The independent effects of these ecological stressors are well studied, however, we lack understanding of their cumulative effects, which may be additive, antagonistic or synergistic. For example, the impacts of biological invasions are often determined by environmental context, which suggests that the effects of invasive species may vary with other stressors such as pollution or climate change. This study examined the effects of an invasive seaweed (*Sargassum muticum*) on the structure and functioning of a synthetic macroalgal assemblage and tested explicitly whether these effects varied with nutrient enrichment and ocean warming. Overall, the presence of *S. muticum* increased assemblage productivity rates and warming altered algal assemblage structure, which was characterised by a decrease in kelp and an increase in ephemeral green algae. The effects of *S. muticum* on total algal biomass accumulation, however, varied with nutrient enrichment and warming, producing antagonistic cumulative effects on total algal biomass accumulation. These findings show that the nature of stressor interactions may vary with stressor intensity and among response variables, which leads to less predictable consequences for the structure and functioning of communities.

There are persistent increases in the range and intensity of anthropogenic stressors exerted on the natural environment (Vitousek et al. 1997), despite efforts to mitigate these impacts through legislation such as the EU Habitats Directives (EU 1992) and the Australian Environment Protection and Biodiversity Conservation Act 1999. Loss of biodiversity is thought to be driven by ecological stressors such as habitat loss and fragmentation (Pimm and Raven 2000), species invasions (Simberloff et al. 2013), eutrophication (Smith et al. 1999), global warming (Schiel et al. 2004) or even ocean acidification (Kroeker et al. 2010). The individual impacts of such stressors on certain species or whole communities have been documented (reviewed by Kroeker et al. 2010 and Thomsen et al. 2011), however, multiple stressors often occur simultaneously (Crain et al. 2008). Multiple stressors may have simple additive effects on ecosystems, which could be predicted from previous studies of independent stressor effects (Kroeker et al. 2010, Dossena et al. 2012). Alternatively, stressors may interact with each other non-additively, leading to a cumulative impact that is either greater than (synergistic) or less than (antagonistic) the sum of the independent effects (Crain et al. 2008). Results from recent meta-analyses suggest that non-additive interactions are common and the nature of such interactions

can vary with environmental context, such as habitat type or climatic conditions (Crain et al. 2008, Darling and Côté 2008). Currently, synergistic cumulative impacts are predicted to be most common, driven by the de-stabilizing effect of a primary stressor that reduces resistance of ecosystems to other stressors, thus, leading to 'ecological surprises' and potentially rapid declines in biodiversity and ecosystem functioning (Paine et al. 1998, Doak et al. 2008). Uncertainty surrounding the cumulative impacts of stressors and the context-dependency of potential interactions among two or more stressors demands more robust experimental work to disentangle such cumulative effects (Crain et al. 2008).

Coastal ecosystems are among the most economically valuable (Barbier et al. 2011) and heavily exploited (Lotze et al. 2006). Among the most ubiquitous stressors in coastal ecosystems are nutrient enrichment, the spread of invasive species and global warming (Lotze et al. 2006). Individually, these stressors can degrade habitats (Smith et al. 1999), change species distributions and assemblage structures (Dossena et al. 2012), and alter ecosystem processes (Thomsen et al. 2011). Non-additive interactions may occur where these stressors influence ecosystems simultaneously and lead to complex cumulative impacts (Mckee et al. 2002,

0 Kratina et al. 2012). For example, nutrient enrichment may amplify an increase in productivity and growth rates induced by warming, by reducing the metabolic constraints associated with nutrient supply (Tadonl    2010).

5 Previous research has focussed on interactions among nutrient enrichment, climate change and species loss (Blake and Duffy 2010, O'Connor and Donohue 2013), while interactions with another common biotic stressor, invasive species, have been less well studied (Strayer 2012). Impacts of invasive species, including the replacement of native species and alteration of ecosystem process rates, are often context-dependent (Thomsen et al. 2011), which may drive different responses between invaded and non-invaded communities to other stressors (Olabarria et al. 2013). Experimental manipulation is required to test how the presence of an invasive species alters community responses to other stressors and to test clearly for potential additive, antagonistic or synergistic interactions among stressors. This study aimed to separate the cumulative effects of three common stressors on the structure and functioning of synthetic assemblages (Diaz et al. 2003) representative of a coastal ecosystem. We tested explicitly for non-additive cumulative effects of: 1) the presence of an invasive algal species (*Sargassum muticum*); 2) nutrient enrichment; and 3) ocean warming, on assemblage productivity, algal biomass accumulation and assemblage structure of a synthetic macroalgal assemblage.

Methods

30 This experiment ran for six weeks during June–July 2012 in outdoor flow-through mesocosms at Queen's Univ. Marine Laboratory, Portaferry, Co. Down, Northern Ireland. The mesocosm array consisted of ten sea water tables, each containing eleven 45-l mesocosms supplied with sand filtered seawater direct from the adjacent Strangford Lough. Shallow coastal benthic assemblages were created to mimic rock pool communities on local shores, which are typical of the region and based on local field surveys. Synthetic assemblages comprised of six algal species (*Fucus serratus*, 21 g wet biomass; *Laminaria digitata*, 17 g; *Corallina officinalis*, 16 g; *Mastocarpus stellatus*, 3 g; *Cladophora* sp., 2 g; *Ulva lactuca*, 1 g), which were present in proportional abundance and biomass of each species in rock pools of similar size to the mesocosms. Algae were cleared of epiphytes manually and insecticide was used to remove unwanted epibiota. Algae were attached to plastic mesh (20 mm mesh size) in each mesocosm and seawater was aerated and renewed by dump buckets (approx. mean flow-through rate: 4 l min⁻¹) simulating wave action on rocky shores. Grazing gastropods were added to each mesocosm to incorporate a realistic grazing pressure on the algae based on surveys of the most abundant grazers in local rock pools. These were the limpet *Patella vulgata* (2.27 individuals m⁻¹), winkles, *Littorina littorea* (3.23 individuals m⁻¹) and topshell, *Gibbula umbilicalis* (4.63 individuals m⁻¹). Thus, typical grazing pressure was incorporated into the experimental design and each mesocosm had two limpets (dry biomass: 10.51 ± 0.44 g, mean ± S.E.), three winkles (7.53 ± 0.15 g) and four topshells (3.72 ± 0.08 g).

Experimental design and set-up

The three factors manipulated in the experiment were: 1) presence of an invasive species (*Sargassum muticum*; fixed, two levels: invaded, non-invaded); 2) nutrient concentration (fixed, three levels: ambient, N+, N++); and 3) water temperature (fixed, three levels: ambient, T+, T++), yielding eighteen experimental treatments and ninety experimental units (n = 5, of all possible combinations). This factorial design allowed the independent and interactive effects of each of the stressors (all three factors at each level) to be tested. Treatments were allocated randomly to mesocosms, which were rearranged approximately weekly to minimise location artefacts (Bruno and O'Connor 2005).

The presence of an invasive species was manipulated by the addition of *S. muticum* (11 g). This furoid algae, originating in northeast Asia, is invasive in northwest Europe and became established in Strangford Lough in 1995 (Boaden 1995). *Sargassum muticum* is a strong competitor with native species and a driver of community structural changes (White and Shurin 2011). In non-invaded treatments, *S. muticum* was substituted with the morphologically similar (Wernberg et al. 2000) native furoid *Halidrys siliquosa* (11 g) to control for biomass addition, thus, all synthetic assemblages contained similar total algal biomass (60 g) at the beginning of the experiment.

In nutrient enriched treatments, the inorganic nitrogen and phosphorus content of the mesocosms was elevated using slow release fertilizer pellets (11 N: 11 P: 18 K) contained in 50 ml nutrient diffusers (70 g in N+ and 140 g in N++ treatments) similar to Worm et al. (2000). Non-enriched treatments contained empty diffusers to limit experimental artefacts. To ensure that nutrient enrichment treatments were effective, water samples were taken after four weeks and analysed using an autoanalyzer. Water samples from enriched treatments had significantly greater concentrations of dissolved inorganic nitrogen (DIN), phosphorus and ammonium (Kruskal–Wallis test with multiple comparisons, ambient < N+ < N++, DF = 2, p < 0.001). Ambient nutrient enrichment treatments contained 1.63 ± 0.07 µm l⁻¹ DIN, 0.38 ± 0.01 µm l⁻¹ phosphate and 0.68 ± 0.03 µm l⁻¹ ammonium (mean ± SE), typical of low summer nutrient concentrations in this region (Hydes et al. 1999). Medium nutrient enrichment (N+) raised DIN content by 0.89 ± 0.224 µm l⁻¹, phosphate content by 0.191 ± 0.018 µm l⁻¹ and ammonium content by 0.533 ± 0.044 µm l⁻¹ above ambient, while high nutrient enrichment treatments (N++) elevated DIN by 1.112 ± 0.112 µm l⁻¹, phosphate content by 0.376 ± 0.024 µm l⁻¹ and ammonium content by 1.007 ± 0.091 µm l⁻¹ above ambient. This increase of around 70% is in line with previous nutrient enrichment studies (Worm et al. 2000, Sugden et al. 2008, Canning-Clode et al. 2008).

There were also three levels of seawater temperature in the experiment: ambient, medium (T+) and high (T++). Mesocosms were warmed as required using submersible aquarium heaters (300 watt). Water temperature was increased by 1.86 ± 0.04°C (T+; mean ± SE) and 2.44 ± 0.05°C (T++) above ambient temperature (13.04 ± 0.03°C) during the experiment, which mimics current climate change predictions for this region (IPCC 2007) and tracked

0	natural variation. Water temperature within each mesocosm was monitored during the experiment and warming treatments were effective (mixed effects model, fixed factor: temperature treatment, random factor: mesocosm, with Tukey post hoc comparisons, ambient < T+ < T++, DF = 2, $p < 0.001$, Supplementary material Appendix 1).	61
5		65
	Response variables	
10	Gross assemblage productivity was estimated from dissolved oxygen concentrations using an optical probe after periods of artificially induced darkness and subsequent similar periods of sunlight (Nielsen 2001, Noël et al. 2010). Initial oxygen concentration was measured and then mesocosms were immediately covered with a lid and subject to a dark incubation period of 90–120 min to estimate respiration.	70
15	After incubations, a second set of measurements for oxygen concentration were taken. A third set of measurements were then taken after a further 20–70 min light period that allowed photosynthesis to resume. Dark incubation periods were varied to ensure that a quantifiable change in dissolved oxygen concentration was achieved and light incubation periods were varied to avoid oxygen super-saturation (Noël et al. 2010). Gross assemblage productivity was calculated from the sum of estimates of net assemblage productivity and assemblage respiration. Productivity measurements were standardised for algal dry biomass (Griffin et al. 2010). Photosynthetically active radiation (PAR) was recorded during oxygen concentration measurement and mean PAR was $1345.41 \pm 18.46 \mu\text{E s}^{-1} \text{ m}^{-2}$ (mean \pm SE, $n = 270$).	75
20	Irradiance levels were above those recorded as saturating light in similar rock pool assemblages ($600 \mu\text{mol photons s}^{-1} \text{ m}^{-2}$, Arenas et al. 2009).	80
25	Dry biomass (dried at 60°C until a constant biomass) of each algal species was quantified at the end of the experiment to test for differences in algal assemblage structure and biomass accumulation among treatments. Total algal biomass accumulation was calculated as the sum of the difference between dry biomass at the beginning and the end of the experiment. Algal dry biomass at the start of the experiment was derived from wet-dry biomass relationships established for all species through the collection and drying (60°C until a constant biomass) of additional wet biomass samples.	85
30		90
35		95
40		100
	Data analysis	
45	Three-way analysis of variance (ANOVA) was used to test for non-additive cumulative effects among treatments on gross assemblage productivity and total algal biomass accumulation. This approach was utilised as interaction terms within an ANOVA test for deviations from the additive model and allow antagonistic and synergistic interactions among stressors to be identified (Billick and Case 1994, Dunne 2010). To preserve the integrity of the experimental procedure, eighteen (out of ninety) experimental units were excluded from the analysis because precise grazer densities could not be assured for the duration of the experiment. This led to some uneven replication of treatments, however, all treatments were replicated a minimum of three times. To account for unequal replication of some treatments, type III sum of squares were used to calculate F-statistics. ANOVAs included	105
50		110
55		115
60		121
	the fixed factors invasion, nutrient enrichment and water temperature and included all possible interactions among these terms. Models were checked for homogeneity of variances using plots of standardised residuals versus fitted values as well as standardised residuals versus the explanatory variables used in each model and QQ-plots and histograms were used to test for normality in conjunction with Shapiro–Wilk and Levene’s tests. Gross assemblage productivity was transformed (cube root) to meet assumptions of normality and heteroscedacity. Post hoc pairwise t -tests (Tukey HSD) were used to make comparisons among levels of significant terms. Data were visualised using means and error (95% confidence intervals) plots to reflect the construct of the ANOVA and add clarification to the results, which is an accepted and informative approach to visualise results of multiple stressor studies (Blake and Duffy 2012, O’Gorman et al. 2012, Burnell et al. 2013). Permutational multivariate analyses of variance (PERMANOVA; Anderson 2001) was used to test hypotheses involving algal assemblage structure, based on Bray–Curtis resemblance matrices calculated from algal dry biomass data with 9999 permutations of residuals under a reduced model. This permutation method was chosen because it reduces type I error for multifactorial analysis (Anderson et al. 2008). Algal dry biomass data were fourth root transformed for multivariate analysis to meet assumptions of homogeneity of dispersions and to reduce the influence of the most abundant species on assemblage structure (Clarke and Warwick 2001). The dry biomass of <i>S. muticum</i> and <i>H. siliquosa</i> were not included in this analysis to distinguish the responses of other species to the presence of <i>S. muticum</i> from the response of <i>S. muticum</i> itself. Where significant differences were detected, post hoc pairwise t -tests were used to make comparisons among levels of significant terms. Similarity of percentages (SIMPER) analyses were conducted to test for individual algal species contributions to overall differences in assemblage structure (Clarke and Warwick 2001). The magnitude of all treatment effects were obtained through the calculation of generalised omega-squared (ω^2) effect sizes (Olejnik and Algina 2003). Multivariate analyses were undertaken in PRIMER ver. 6 (Clarke and Gorley 2006) and univariate analyses were undertaken in R ver. 2.14.2 (R Development Core Team 2011.)	
	Results	
	The presence of <i>Sargassum muticum</i> increased gross assemblage productivity, however, the effect size was small (Table 1a, Fig. 1a). There were no effects of nutrient enrichment or warming and no interactive effects among any of the stressors on gross assemblage productivity (Table 1a, Fig. 1). The presence of the invasive alga <i>S. muticum</i> altered total algal biomass accumulation, however, this effect changed with nutrient concentration and temperature (Table 1b, Fig. 2). Post hoc tests showed that in the absence of <i>S. muticum</i> , total algal biomass accumulation was significantly lower in the medium nutrient treatment compared to ambient and high nutrient treatments (Fig. 2a). Whereas, when <i>S. muticum</i> was present there was no effect of nutrients on algal biomass accumulation (Fig. 2a). In addition, the response of algal biomass accumulation to increasing water temperature varied	

Table 1. Tests for the effects of the presence of an invasive species, nutrient enrichment and warming on assemblage responses: (a) ANOVA with type III sums of squares for effects of the treatments on gross assemblage productivity after six weeks; (b) ANOVA with type III sums of squares for effects of the treatments on total algal biomass accumulation after six weeks, and (c) PERMANOVA based on Bray–Curtis dissimilarity matrix calculated from algal dry biomasses (fourth root transformed to standardise individual species contributions) with 9999 permutations of residuals under a reduced model. Significant terms are highlighted in bold. ω^2 -values are generalised omega-squared effect sizes. Negative ω^2 are shown as zeros.

Source	DF	(a) Gross assemblage productivity				(b) Total algal biomass accumulation				(c) Algal assemblage structure			
		MS	F	p	ω^2	MS	F	p	ω^2	MS	F	p	ω^2
Invasion (Inv)	1	0.040	6.483	0.014	0.071	6.188	0.769	0.384	0.000	19.634	0.635	0.610	0.000
Nutrient enrichment (Nut)	2	0.003	0.456	0.636	0.000	54.817	6.810	0.002	0.139	32.941	1.066	0.389	0.000
Temperature (Temp)	2	0.002	0.391	0.678	0.000	25.022	3.109	0.053	0.055	77.081	2.494	0.029	0.039
Inv \times Nut	2	0.002	0.307	0.737	0.000	43.360	5.387	0.007	0.108	66.197	2.142	0.056	0.031
Inv \times Temp	2	0.001	0.146	0.865	0.000	26.178	3.252	0.046	0.058	15.114	0.489	0.811	0.000
Nut \times Temp	4	0.004	0.624	0.647	0.000	1.728	0.215	0.929	0.000	22.456	0.727	0.713	0.000
Inv \times Temp \times Nut	4	0.008	1.316	0.276	0.017	4.393	0.546	0.702	0.000	8.677	0.281	0.983	0.000
Residuals	54	0.006				8.049				30.907			

between non-invaded and invaded assemblages, however, this effect was comparatively small compared to the interaction between invasion and nutrient enrichment (Table 1b). In the absence of *S. muticum*, algal biomass reduced with warming of 2.4°C compared to ambient and medium temperature treatments (Fig. 2b). There was no effect of temperature on total algal biomass when *S. muticum* was present (Fig. 2b). As such, this invasive species appears to provide a form of resistance to the effects of nutrient enrichment and, to a far lesser extent, warming on total algal biomass accumulation. Increasing temperature affected algal assemblage structure (Table 1c). Specifically, assemblage structure differed in high warming treatments compared to both medium and ambient treatments ($T++ \neq A$; $t = 1.919$, $p = 0.045$; $T++ \neq T+$; $t = 1.640$, $p = 0.047$; $A = T+$; $t = 0.489$, $p = 0.873$, nMDS: Supplementary material Appendix 2). This shift in

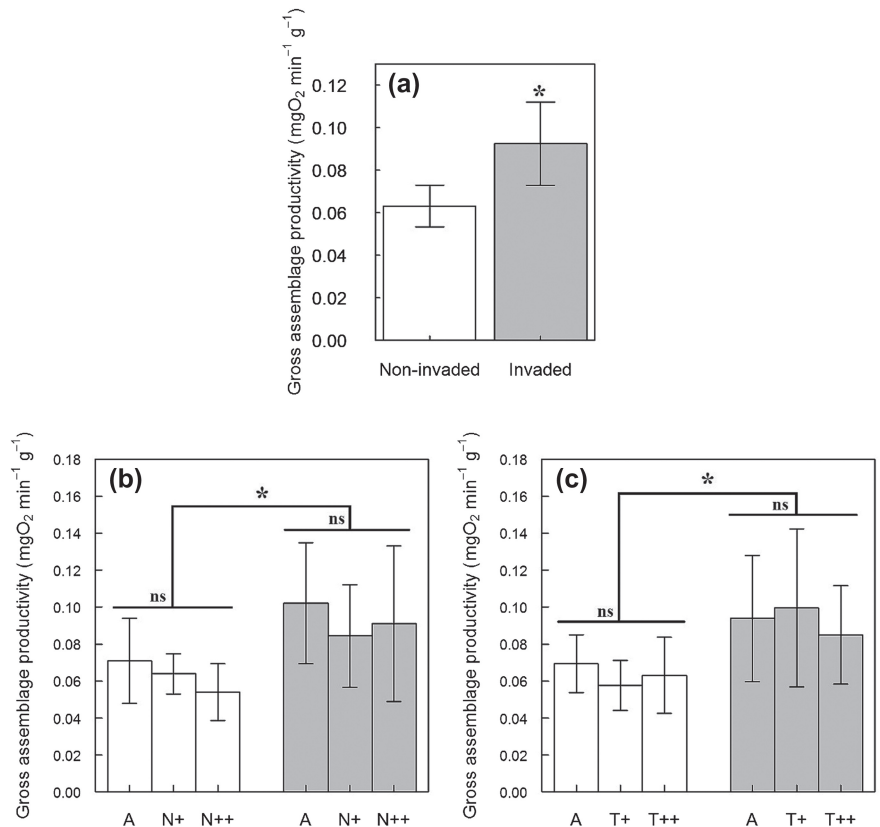


Figure 1. Means (\pm 95% confidence interval) of gross assemblage productivity in experimental treatments following the construct of comparisons within the three factor ANOVA. (a) Gross assemblage productivity in all treatments with the presence and absence of *Sargassum muticum* (analysis is based on 33 invaded and 39 non-invaded replicates as temperature and nutrient enrichment treatments were pooled); (b) Gross assemblage productivity in each of the nutrient enrichment treatments (each bar represents between $n = 11$ and $n = 14$ replicates as temperature treatments were pooled); (c) Gross assemblage productivity in each of the temperature treatments (each bar represents between $n = 10$ and $n = 14$ as nutrient enrichment treatments were pooled). Non-invaded treatments are denoted by open bars and invaded treatments are denoted by grey bars. * denotes groups of treatments that differ significantly ($p < 0.05$).

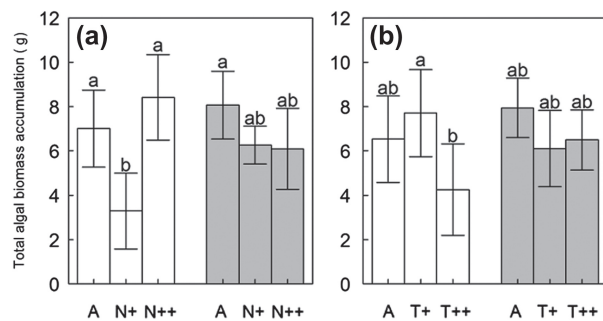


Figure 2. Means (\pm 95% confidence interval) of total algal biomass accumulation in treatments following the construct of comparisons within the three factor ANOVA. Total algal biomass accumulation in (a) each nutrient enrichment treatment (each bar represents between $n = 11$ and $n = 14$ replicates as temperature treatments were pooled) and (b) each temperature treatment (each bar represents between $n = 10$ and $n = 14$ replicates as nutrient enrichment treatments were pooled). Non-invaded treatments are denoted by open bars and invaded treatments are denoted by grey bars. Letters indicate treatments that are statistically indistinguishable from each other based on post hoc tests ($p > 0.05$).

algal assemblage structure was driven generally by an increase in the green alga, *Ulva lactuca* (Fig. 3f) and reduction in biomass of the brown alga *Laminaria digitata* (Fig. 3b) in warmer treatments compared to cooler treatments (Table 2). None of the other species showed strong responses to warming (Fig. 3a, c–e). There were no effects of species invasion or nutrients on algal assemblage structure (Table 1c).

Discussion

By manipulating the presence of an invasive species (*Sargassum muticum*), nutrient enrichment and warming simultaneously, we have shown that, although the impact of invasion can be independent (e.g. assemblage productivity), the response of the invaded community to other stressors can also differ from non-invaded communities (e.g. algal biomass accumulation). Our findings illustrate the non-additive cumulative effects of multiple stressors. However,

Table 2. SIMPER analysis identifying algal species that contributed to dissimilarities in assemblage structure in response to warming treatments including comparisons between: (a) ambient conditions and medium warming (mean dissimilarity = 6.31%); (b) medium and high warming (mean dissimilarity = 7.31%); and (c) mean dissimilarity between ambient and T++ = 7.83%.

Species	Mean biomass (g)		Diss/SD	Contribution (%)
(a)	A	T+		
<i>Ulva lactuca</i>	0.153	0.290	1.24	42.31
<i>Corallina officialis</i>	6.787	6.558	1.18	15.09
<i>Cladophora sp.</i>	0.800	0.786	0.67	14.14
<i>Laminaria digitata</i>	5.911	6.068	1.34	12.85
<i>Fucus serratus</i>	8.582	9.000	1.36	8.44
<i>Mastocarpus stellatus</i>	1.456	1.518	1.36	7.17
(b)	T+	T++		
<i>Ulva lactuca</i>	0.290	0.323	1.23	35.04
<i>Laminaria digitata</i>	6.068	3.867	0.94	28.95
<i>Corallina officialis</i>	6.558	6.372	1.07	13.36
<i>Cladophora sp.</i>	0.786	0.846	1.29	9.18
<i>Mastocarpus stellatus</i>	1.518	1.647	1.34	6.90
<i>Fucus serratus</i>	9.000	8.591	1.00	6.57
(c)	A	T++		
<i>Ulva lactuca</i>	0.153	0.323	1.24	33.99
<i>Laminaria digitata</i>	5.911	3.867	0.88	25.80
<i>Corallina officialis</i>	6.787	6.372	1.16	13.19
<i>Cladophora sp.</i>	0.800	0.846	0.72	12.74
<i>Fucus serratus</i>	8.582	8.591	1.16	7.39
<i>Mastocarpus stellatus</i>	1.456	1.647	1.37	6.89

interactions were only present for one of the processes quantified in the experiment and, importantly, these effects were determined by stressor intensity.

The presence of *S. muticum* led to a small, but statistically significant, increase in gross assemblage productivity, thus, providing support for the theory that invasive species can have wider ranging impacts beyond effects on single species (Simberloff et al. 2013). The mechanism behind this increased productivity in invaded assemblages may be associated with differences in the functional traits, such as phenology and growth rates, of *S. muticum* compared to the morphologically similar native species, *Halidrys siliquosa*. Although both species are considered perennial, the phenology of *S. muticum* and *H. siliquosa* differ (Wernberg et al.

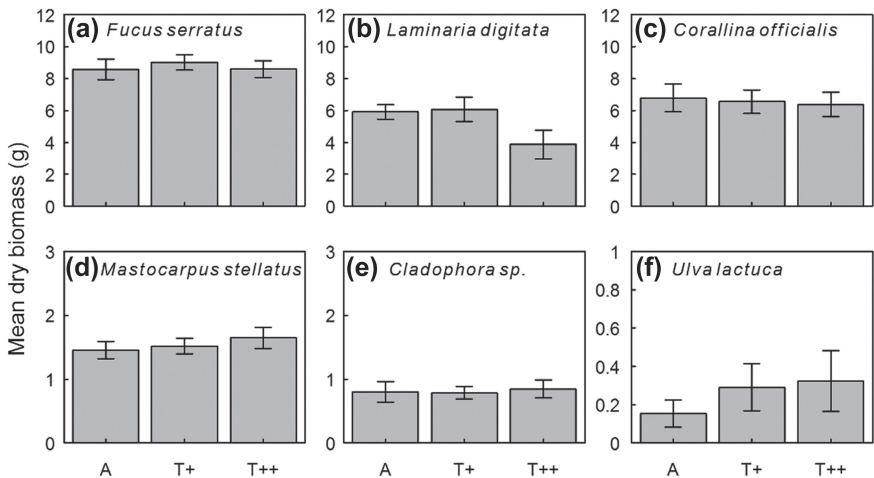


Figure 3. Means (\pm 95% confidence interval) of algal species dry biomass after six weeks in each of the temperature treatments (A: $n = 22$, T+: $n = 24$, T++: $n = 26$ as nutrient enrichment and invasion treatments were pooled).

2000). This experiment was conducted in summer when northeast Atlantic populations of *S. muticum* undergo a rapid growth phase, whereas *H. siliquosa* does not show any seasonal variation in growth (Wernberg et al. 2000). The rapid seasonal growth rate of *S. muticum* is thought to be supported by increased rates of photosynthesis rather than by the use of stored carbon, suggesting that higher photosynthetic rates may occur in this period when compared to winter months (Lewey and Gorham 1984). This rapid growth can cause *S. muticum* to dominate algal assemblages, constraining light availability, which may reduce other species photosynthetic ability and lead to declines in the abundance of underlying algae (White and Shurin 2011). Interestingly, in this study the presence of *S. muticum* did not alter assemblage structure suggesting that this process did not occur and indicating that although *S. muticum* increased rates of productivity, this was not to the detriment of other species. It is possible that the lack of a dominance effect on assemblage structure may be because of the short duration of the study, or owing to differences in limiting resources between natural and mesocosm systems (Stachowicz et al. 2008).

We did not detect any effects of warming or nutrient enrichment on assemblage productivity rates, however, warming altered assemblage structure through increases in biomass of the ephemeral green alga, *Ulva lactuca* and declines in the biomass of the brown alga, *Laminaria digitata* in treatments with a warming of 2.4°C. Although statistically the effect size was small, this change in assemblage structure is of notable ecological interest owing to the importance of *L. digitata* for the functioning of coastal ecosystems. *Laminaria digitata* is a kelp species that thrives in colder waters and is thought to be near the southern limit of its geographical range on these shores (Smale et al. 2013). Kelp is a highly productive component of coastal ecosystems, supporting diverse communities and facilitating the provision of many coastal goods and services (Smale et al. 2013). Our findings support concerns that global warming may threaten kelp species, which are at the edge of their range, potentially causing range contraction and declines in associated ecosystem services (Smale et al. 2013). There was no reduction in assemblage productivity associated with the decline in biomass of *L. digitata* under warmed conditions, indicating that the corresponding increase in *U. lactuca* may have compensated in terms of maintaining assemblage productivity. Ephemeral algae, such as *U. lactuca*, have previously been shown to proliferate in increased warming scenarios (Schiel et al. 2004). Although *L. digitata* and *U. lactuca* are not functionally equivalent (Littler 1980), increases in *U. lactuca* biomass and productivity may have compensated for the decline of *L. digitata* and therefore account for the lack of an effect of warming on overall assemblage productivity. A similar compensation effect has been identified from recent field experiments where the proliferation of ephemeral species maintained community productivity after the loss of canopy species (Crowe et al. 2013). It has been shown that shifts in algal assemblage structure can maintain processes such as total biomass and production at similar rates, however, different algal species are rarely functionally equivalent in terms of multiple ecosystem properties, thus, true compensation cannot be inferred (Bruno et al. 2005, O'Connor and Bruno 2007).

In terms of the cumulative effects of stressors, we identified non-additive interactive effects on total algal biomass accumulation, however, contrary to previous work (Staeher and Sand-Jensen 2006), we found no evidence of an interaction between nutrient enrichment and warming on assemblage productivity. Nutrient enrichment could be expected to enhance the effects of warming on assemblage productivity through reduction of potential nutrient limitation (Staeher and Sand-Jensen 2006). However, our results suggest that nutrient supply may not be a limiting factor in this system, because there were no effects of nutrient enrichment on productivity rates or assemblage structure.

We have demonstrated how the effect of a stressor (e.g. nutrient enrichment) on total algal biomass accumulation was determined by the presence of a second stressor (e.g. an invasive species). For example, the decrease in algal biomass accumulation in medium nutrient enriched treatments seen in non-invaded assemblages, did not occur when the second stressor, the invasive species *S. muticum*, was present. This suggests that an antagonistic interaction occurred between these stressors. Antagonistic interactions have been identified between other pairs of anthropogenic stressors, such as between nutrient enrichment and ocean acidification (Burnell et al. 2013). In our study, total algal biomass accumulation was similar across treatments when *S. muticum* was present, whereas in non-invaded assemblages under medium nutrient enrichment and under high warming treatments there was a decline in algal biomass accumulation. The antagonistic interaction between the presence of *S. muticum* and warming effects on algal biomass accumulation may have been driven by differences in the responses of *H. siliquosa* and *S. muticum* to warming. In non-invaded assemblages, *H. siliquosa* may have a reduced fitness in high warming treatments as water temperatures in these treatments were at the upper limits experienced typically by this species during the summer and, thus, could have led to a decrease in algal biomass accumulation in the non-invaded assemblages (Morris and Taylor 1983). In contrast, the positive effect of *S. muticum* on total algal biomass accumulation suggests that *S. muticum* is better adapted to take advantage of warmer water temperatures (Walther et al. 2009). Invasive species may have different tolerances to environmental variables, such as temperature, as a consequence of their different evolutionary environments (Byers et al. 2002). This rationale could explain why invasive species are sometimes better adapted to survive and respond successfully to opportunities driven by anthropogenic environmental change, such as increased resource availability from declines in native species (MacDougall and Turkington 2005).

We have shown that in non-invaded assemblages, total algal biomass accumulation declined in medium nutrient enrichment treatments. The mechanism behind this non-additive effect is unclear, however, it is typical of the type of unpredictable results found when multiple stressors occur (Paine et al. 1998). One speculative explanation is that nutrient enrichment may have stimulated algal growth and increased levels of nitrogen in algal tissues through the storage of excess nutrients (Pedersen and Borum 1996). This could have provided a higher quality food source for grazing

0 gastropods and enhanced grazing activity, which may have
 constrained algal biomass accumulation (Boyer et al. 2004).
 At the high levels of enrichment, algal growth may have been
 enhanced to a point where the bottom-up effects of nutrient
 enrichment were greater than the top-down grazing effects
 5 leading to greater biomass accumulation than in medium
 nutrient enrichment treatments. Although this explana-
 tion is speculative because specific grazing rates (top-down
 control) was not quantified as part of this study, direct and
 indirect effects of different levels of nutrient enrichment may
 10 be a contributing factor to the differences in the importance
 of bottom-up and top-down control on algal growth and
 biomass as found in previous studies (Valdivia et al. 2008,
 Atalah and Crowe 2010). The current study is one of the
 few that has manipulated nutrient enrichment over more
 15 than two levels (but see Sugden et al. 2008, Canning-Clode
 et al. 2008). Many previous studies crossing a wide range
 of systems have tested for a presence or absence effect of
 nutrient enrichment (Blake and Duffy 2010, O'Connor and
 Donohue 2013). These studies have allowed generalisations
 20 to be formed (Smith et al. 1999, Sala and Knowlton 2006),
 however, there is no consideration of stressor effects occur-
 ing along an intensity gradient. Our study tested a range of
 nutrient enrichment and warming scenarios and highlights
 that there are more than simple presence or absence effect
 25 of stressors, which further complicates the prediction of
 interactive cumulative effects (Steudel et al. 2012).

The presence of antagonistic cumulative effects of multiple
 stressors on total algal biomass accumulation, but not on
 other assemblage responses (i.e. productivity), further high-
 30 lights the unpredictable effects of multiple stressors on eco-
 system functioning. Our findings provide strong empirical
 evidence of non-additive interactions among stressors, which
 is consistent with conclusions of recent meta analyses, indi-
 cating that antagonistic (Crain et al. 2008, Darling and Côté
 35 2008), rather than synergistic cumulative effects are most
 prevalent (Sala and Knowlton 2006, Halpern et al. 2007).
 These non-additive interactions between the presence of
 the invasive species and other stressors exemplify how inva-
 sive species have a high potential for complex interactions
 40 owing to their context-dependent effects on biodiversity and
 ecosystem functioning (Thomsen et al. 2011, Simberloff
 et al. 2013).

To conclude, the presence of an invasive species affected
 functioning through both independent effects and non-
 45 additive interactive effects with other stressors. In this study,
 non-additive interactive effects were determined by stressor
 intensity and varied depending on the ecosystem process.
 The unpredictable nature of these interactions highlights the
 importance of considering a range of ecosystem processes
 50 and more importantly, gradients of environmental contexts
 or stressor intensities when aiming to identify, predict and
 mitigate the wider impact of multiple stressors on ecosystem
 functioning and services.

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